

Appln No.: 09/601,644  
Amendment Dated: April 26, 2006  
Reply to Office Action of February 27, 2006

**Amendments to the Claims:**

This listing of claims will replace all prior versions, and listings, of claims in the application:

**Listing of Claims:**

1. (currently amended) A method for making a cytotoxic mutant protein or pool of proteins from a cytotoxic wild type protein, said mutant protein or pool of proteins having a different receptor-binding specificity for a receptor that is different from the receptor to which ~~than~~ the wild type protein has receptor binding specificity, comprising:

(A) selecting a heteromeric protein toxin having a toxic domain or subunit and a binding domain or subunit, wherein the heteromeric protein toxin is a ribosome inactivating protein;

(B) incorporating mutations into DNA encoding the binding domain or subunit of the heteromeric protein toxin to produce a plurality of variant forms of the heteromeric protein toxin;

(C) generating a library of microorganism clones producing variant forms of the heteromeric protein toxin;

(D) screening the variant forms of the heteromeric protein toxin of said library against a population of screening cells by (i) isolating clones or pools of clones producing said variant forms of the heteromeric protein toxin. (ii) treating preparations of said population of screening cells with variant forms of the heteromeric protein toxin produced by the isolated clones or pools of clones, (iii) observing the treated preparations of said population of screening cells for toxicity, and (iv) selecting based on the observation of toxicity a cytotoxic mutant protein or pool of cytotoxic mutant proteins that inhibits or kills said population of screening cells to a greater extent than the wild-type cytotoxic protein, whereby said selected mutant protein or pool of proteins has the a different receptor binding specificity than the wild-type binding protein, wherein the screening cells are insensitive to the selected cytotoxic heteromeric protein toxin at a concentration used in the screening; and

(E) making additional copies of the selected cytotoxic mutant protein or pool of proteins.

2. (previously presented) The method of claim 1, wherein the cells in the population of screening cells are eukaryotic.

3. (original) The method as claimed in claim 1, wherein said library comprises bacteria or bacterial supernatants containing said variant protein toxins.

4. (original) The method as claimed in claim 1, wherein said library comprises yeast or yeast supernatants containing said variant protein toxins.

Appln No.: 09/601,644

Amendment Dated: April 26, 2006

Reply to Office Action of February 27, 2006

Do Not  
Enter  
mg

cells for toxicity, and (iv) selecting based on the observation of toxicity a cytotoxic mutant protein or pool of cytotoxic mutant proteins that inhibits or kills said population of screening cells to a greater extent than the wild-type cytotoxic protein, whereby said selected mutant protein or pool of proteins has the a different receptor binding specificity than the wild-type binding protein, wherein the screening cells are insensitive to the selected wild-type cytotoxic heteromeric protein toxin at a concentration used in the screening; and

(E) making additional copies of the nucleic acid sequence or pool of nucleic acid sequence encoding the selected cytotoxic mutant protein or pool of cytotoxic mutant proteins.

38. (previously presented, withdrawn)  
the population of screening cells are eukaryotic.

The method of claim 37, wherein the cells in

39. (previously presented, withdrawn)  
the population of screening cells are tumor cells.

The method of claim 38, wherein the cells in

40. (previously presented, withdrawn)  
cells are breast cancer cells.

The method of claim 39, wherein the tumor

41. (previously presented, withdrawn)  
binding domain or subunit is derived from the B-subunit of either Shiga toxin and Shiga-like toxins, or homologous counterparts from *E. coli* heat labile enterotoxins, cholera toxin, pertussis toxin or the receptor binding domain of ricin.

The method of claim 37, wherein the

42. (canceled)

43. (new) The method of claim 1, wherein in step B the mutations are randomly incorporated into the DNA encoding the binding domain or subunit of the heteromeric protein toxin.